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COMMENTARY

The Potential Role of Smartphone-Based Microfluidic Systems for Rapid Detection of COVID-19 Using Saliva Specimen

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In late 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) disease (COVID-19) emerged in Wuhan, China, and is a current life-threatening concern worldwide [1]. Rapid and accurate diagnostic tests can control this outbreak in public and healthcare facilities [2]. However, many modern and costly medical diagnostic technologies are not available to people in developing countries and resource-limited regions [3].

Point-of-care testing (POCT) is described as the use of easily portable devices to carry out analytical testing outside of the central laboratory [4]. POCT is crucial for managing human healthcare since it affords an early and fast diagnostic result [5]. Microfluidic systems, which have transformed diagnostic POCT in many medical and dental fields [3, 6], are designed to perform measurements on small amounts of fluids with fairly high sensitivity and speed, without the need for any trained healthcare worker [3].

The employment of microfluidic devices combined with POCT may result in instruments that can provide better healthcare for the general population, particularly considering their cost-effectiveness. Microfluidics is presently used in the detection of virus, disease diagnostics, and microbial studies. Most microfluidics studies have used saliva as a diagnostic fluid. Being appropriate for POCT, saliva has many advantages, since saliva samples can be easily stored and collection is fast, non-invasive, inexpensive, and safe for patients and healthcare providers and does not necessitate skilled personnel [3].

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The application of traditional analytical biosensing instruments is limited since they are comparatively bulky, expensive, and not easy to handle [7].

Smartphone-based microfluidic biomedical sensory systems combine smartphones, microfluidic components, and sensory elements. This combination offers a user-friendly, easily accessible, miniaturized, and portable technology [7].

Different smartphone-based microfluidic biosensor systems, such as imaging biosensors, biochemical sensors, immune biosensors, hybrid biosensors, and molecular sensors, are employed at the point of care (POC) [5].

The smartphone carries out the whole analytical process on biological samples, including data collection, analysis, and result display. It has the aptitude to receive data from sensors and to control various actuators or send data to and receive data from other devices using wires or wirelessly [3].

To obtain, analyze, and deliver data, most of these platforms and technologies depend on electrical or optical detectors that necessitate sophisticated instrumentation and expensive hardware. The image sensors within phone cameras have optimal sensitivity for many diagnostically pertinent purposes [8]. The complementary metal oxide semiconductor (CMOS) camera sensor of a smartphone has been employed for detecting optical signals, including fluorescence for isothermal nucleic acid amplification tests [9].

Smartphone-based imaging and sensing platforms are growing as competent substitutes for overcoming the barrier between medical staff and their patients. Likewise, the implementation of smartphone-based diagnostics for COVID-19 allows for fast disease diagnosis and could have a considerable influence on the epidemiology of the disease since accurate geographic and demographic data can be stored mutually with diagnostic data.

Early clinical diagnosis of COVID-19 is challenging in infected patients since they might remain asymptomatic up to 2 weeks after exposure [1]. The current gold standard test for COVID-19 diagnosis is the quantitative reverse transcription polymerase chain reaction (qRT-PCR) of respiratory

samples such as nasopharyngeal and oropharyngeal swabs; however, collection of these specimens exposes healthcare workers to a high risk of infection [1, 2]. Recently, this virus was detected in self-collected saliva of infected patients, a process that might significantly reduce the risk of healthcare worker exposure to the virus [2]. It is documented that saliva can be a potential specimen for COVID-19 diagnosis through qRT-PCR and viral load monitoring [2].

Utilizing a saliva specimen for COVID-19 has some advantages. This is an easy non-invasive procedure; patients themselves can collect it where there is no isolation room available. This would subsequently reduce the nosocomial transmission of disease, and the results would be available much faster. Moreover, it is a feasible procedure when nasopharyngeal specimen collection is contraindicated [2]. More than 100 biomarkers (DNA, RNA, messenger RNA [mRNA], and proteins) have already been recognized in oral fluid, including cytokines (interleukin-8 [IL-8], IL-1b, and tumor necrosis factor alpha [TNF-α]) [3]. Since alterations in many biomarkers such as immunoglobulins, cytokines, and nucleic acids are diagnostic for COVID-19 [10], fast and accurate detection of these biomarkers by employment of smartphone-based microfluidic systems can be helpful in early diagnosis of COVID-19.

Smartphone microfluidic systems that employ saliva samples as a diagnostic fluid often benefit from colorimetric and luminescence techniques by using the phone camera, dark chambers, and holders to receive information from samples [3].

Although saliva is a preferred biofluid sample for monitoring patients at the POC, using untreated saliva directly on a biosensor can result in interference with the sensor system and experimental errors, since whole saliva is very viscous because of the presence of oral particulate matter and adhesive mucin and may consist of unpredictable particles, such as food residue and glycoprotein content. Processing saliva samples for analysis is extremely difficult; however, it is necessary to remove these impurities, while maintaining the target concentration [11].

Different techniques for pretreatment of saliva have been reported [12]. Yager et al. [13] and Helton et al. [14] have used a microfluidic device (H-filter) that used laminar flow to decrease saliva viscosity and the amount of mucins and glycoproteins in saliva samples. The first step was membrane filtration to remove cells, large debris, and the majority of the mucin glycoproteins. The second step used an H-filter for diffusive extraction of impurities. They removed 97% of mucins and 92% of total proteins, while a significant amount of target analytes was preserved.

A POCT for salivary diagnostics and detection of low levels of biomarkers in saliva should have both high sensitivity and high specificity; however, current detection in saliva entails a compromise between specificity and sensitivity. The accuracy of the POCT using saliva should be compared with traditional laboratory gold standards such as enzyme-linked immunosorbent assay (ELISA) for proteins and polymerase chain reaction (PCR) for nucleic acids. To verify the specificity of the POCT, the effect of variations in saliva content must be examined for an individual under controlled and variable conditions [15].

ELISA, PCR, and reverse transcription loop-mediated isothermal amplification (RT-LAMP) assays are miniaturized onto a chip-based device with potential advantages including speed, price, handiness, throughput, and automation [4, 16, 17]. The effectiveness of smartphone-based microfluidic biosensors has already been demonstrated in healthcare diagnostics [7].

Qiu et al. [18] have described rapid detection of H1N1 in less than 30 min using a smart phone-based microfluidic convection PCR, for which capillary tubes were fabricated with injection molding. The smartphone was employed to take florescent images and analyze their signal intensity.

RT-LAMP is a one-step, PCR-based nucleic acid amplification process that has been employed to diagnose infectious diseases [1]. RT-LAMP has a number of advantages, including high specificity and sensitivity. Additionally, it requires less than 1 h to be completed and can be employed in different ranges of pH and temperature. Moreover, the reagents are comparatively low-priced and are stable at room temperature [1]. A recent study has demonstrated that RT-LAMP can specifically detect COVID-19 in simulated patient samples including saliva in less than 30 min. This new diagnostic method can be done without any training or equipment and also can be used outside of a central laboratory on various types of specimens [1]. Another study has used three RT-LAMP primers for detecting ORF1ab, N, and E genes of SARS-CoV-2. The accuracy rate of detecting these three genes together was 99%, because detecting both ORF1ab and N genes significantly increases the specificity and sensitivity of the test. This simple measurement can lead to rapid and accurate COVID-19 diagnosis [19]. The Zika virus (ZIKV) outbreak in Brazil in 2015 presented severe fetal abnormalities, called congenital Zika syndrome. Studies using different methods have developed smartphone-based microfluidic systems for detecting ZIKV from human complex sample matrices including saliva through RT-LAMP. These methods can easily be adopted to detect other types of viral infections [16, 20].

The diagnosis of viral diseases such as avian influenza has been reported with a sensitivity of 96.5% and specificity of 98.5% employing sandwich ELISA incorporated into fluorescent lateral flow assay (LFA) strips [8, 21]. Additionally, in a study of 82 confirmed and 58 probable cases, combined sensitivity of PCR and immunoglobulin M (IgM) ELISA directed at nucleocapsid antigen was 98.6% compared to 51.9% with a single PCR test. During the first

5.5 days, quantitative PCR had a higher positivity rate than IgM, whereas IgM ELISA had a higher positivity rate after day 5.5 of disease [22].

Since the integration of both smartphones and microfluidic systems has provided a technology that is user-friendly, easily accessible, miniaturized, and portable, using smartphone-based microfluidic PCR or RT-LAMP alongside ELISA has the potential to allow the rapid detection of COVID-19 in patient samples, particularly saliva. The combination of smartphones or tablets with microfluidics will allow continuous and easy health monitoring of individuals or populations during and after COVID-19 outbreaks. Fabricating and developing such fast and accurate devices can effectively equip us to handle current and probably future outbreaks.

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Compliance with Ethical Standards

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